ROBUST SUMMARY

PHYSICAL AND CHEMICAL DATA

1.0 MELTING POINT

Value:	181- 182°C
Decomposition:	Yes [ ] No [X] Ambiguous [ ]
Sublimation:	Yes [ ] No [X] Ambiguous [ ]
Method:	OECD Test Guideline 102 (1993)
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	4-Nitro-N-Methylphthalimide (4-NPI, CAS RN 41663-84-7); Lot UI0044 from General Electric Company; Purity: 96.4% 4-NPI, 3.6% 3-NPI received as a water slurry (~ 60% 4-NPI/40% water).
Remarks:	The melting point (MP) of a calibration substance (phenanthrene) was determined at least in duplicate (using separate heating runs). The MP of the 4-NPI samples (wet and dried) were determined in triplicates (using separate heating runs) as follows: The sample was placed at the sealed end of a glass capillary tube to a height of approximately 2 mm. The tube was placed in the melting point apparatus, the heating block of which was heated to approximately 5 °C below the MP. The contents of the capillary were heated at 1 °C/min and the temperatures of Stages A, D, and E were recorded. Stages B and C were not distinctly observed, and these temperatures could not be recorded. It was also noted if the sample changed color or appeared to decompose during the test. One 4-NPI MP was determined using a heating rate of 10 °C/min, starting at ambient temperature. Moist 4-NPI was ground in a mortar and pestle to < 0.1 mm, as visually assessed. The following was done in duplicate. After grinding, 4-NPI (~ 1 g) was immediately weighed (4-place balance) into a pre-weighed vessel. The sample was dried in a vacuum oven at 88 °C for 17 h at approximately 16 mm Hg (water aspirator). The sample was cooled in a dessicator and weighed to determine the loss on drying. The duplicate samples were stored in separate capped glass vials in a dessicator. Results: The calibration substance, phenanthrene, gave an average melting point of 98 - 99 °C, which, when compared to the expected MP of 99 - 101 °C, indicated a bias of 1 to 2 °C. The MP results for 4-NPI were adjusted 2 °C upward to account for this bias. The observed bias adjusted average MP of the dried samples were 181 - 182 °C, which was higher and sharper than that observed for the wet sample (173 - 178 °C). This difference was probably because of a melting point depression effect caused by moisture in the 4-NPI.
Reference:	Reimer, G.J. (2002). Unpublished report no. SP11291 1419-MP (BC Research Inc. Project no 11291 1419) entitled "Physical/chemical property of 4-Nitro-N-Methylphthalimide (4-NPI), CAS # 41663-84-7: Melting Point (OECD 102)", dated April 15, 2002 for General Electric

	Company, Pittsfield, MA, USA; from BC Research Inc., Vancouver, BC, Canada.
Reliability:	(Klimisch Code 1) Valid without restrictions.

2.0 BOILING POINT

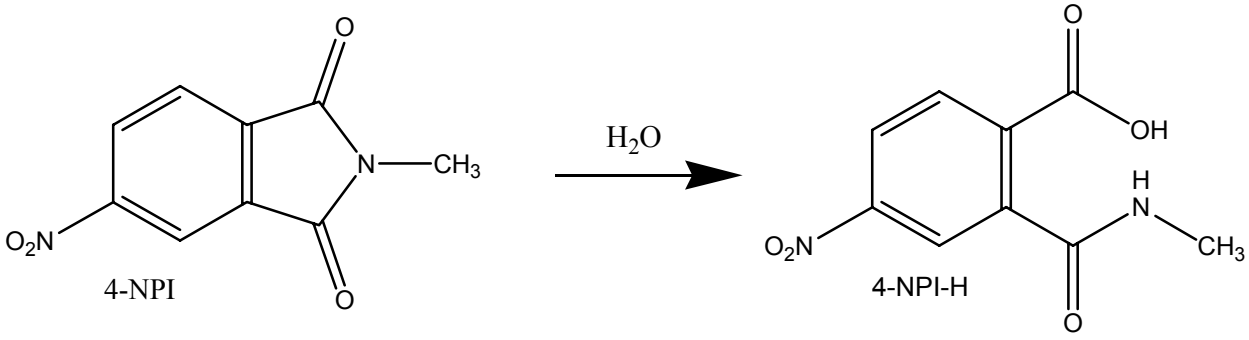
Value:	361 ± 25°C
Pressure:	101.3 kPa
Decomposition:	Yes [ ] No [X] Ambiguous [ ]
Method:	OECD Test Guideline 103 (1993)
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	4-Nitro-N-Methylphthalimide (4-NPI, CAS RN 41663-84-7); Lot UI0044 from General Electric Company; Purity: 96.4% 4-NPI, 3.6% 3-NPI received as a water slurry (~ 60% 4-NPI/40% water).
Remarks:	Samples of 4-NPI (wet and dried) were heated (in quadruplicate) in an open capillary tube from 150 °C to 301 °C in 15 min. During heating, the liquid 4-NPI sample turned from yellow to brown and finally a dark brown solid was observed at 300 °C. No vigorous boiling was observed during heating. Minor bubbling of the sample was observed at approximately 200 °C, and a small amount of condensate (~ 25 % of the sample) was observed at approximately 220 °C in a cooler region of the capillary tube. This minor bubbling diminished at approximately 280 °C. From the recorded observations, it was concluded that the boiling point (BP) of 4-NPI at atmospheric pressure (101.3 kPa) could not be measured because of its thermal degradation. The results for the dried 4-NPI yielded essentially the same results. BP measurement at reduced pressure was not performed because the calculated BP at atmospheric pressure (361 ± 25 °C) was greater than 300 °C, which is the regulatory limit set by the US EPA (EPA, 1999) and Environment Canadian (CEPA, 1993).
Reference:	Reimer, G.J. (2002). Unpublished report no. SP11291 1419-BP (BC Research Inc. Project no. 11291 1419) entitled "Physical/chemical property of 4-Nitro-N-Methylphthalimide [CAS RN 41663-84-7]: Boiling Point (OECD 103)", dated June 6, 2002 for General Electric Company, Pittsfield, MA, USA; from BC Research Inc., Vancouver, BC, Canada.
Reliability:	(Klimisch Code 1) Valid without restrictions.

3.0 VAPOR PRESSURE

No studies were found.

4.0 PARTITION COEFFICIENT ($Log_{10}P_{ow}$)

Log Pow:	1.5 ± 0.3
Temperature:	21ºC
Method:	Calculated [X] Measured [ ] Based on OECD Test Guideline 107 (1993)
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	4-Nitro-N-Methylphthalimide (4-NPI, CAS RN 41663-84-7); Lot UI0044 from General Electric Company; Purity: 96.4% 4-NPI, 3.6% 3-NPI received as a water slurry (~ 60% 4-NPI/40% water).
Remarks:	It was not possible to perform the *n*-octanol/water partition coefficient (P_{ow}) test on 4-NPI because of its likely hydrolysis during the test procedure. The calculated log(P_{ow}) values for 4-NPI and its hydrolysis product (4-NPI-H) were 1.5 ± 0.3 and 0.2 ± 0.4 respectively. The *n*-octanol/water partition coefficient was calculated using computer software manufactured by ACD (Advanced Chemistry Development, Inc., Toronto, Canada). Comparison of ACD-calculated partition coefficients with experimentally observed values, for several different classes of organic compounds, showed correlations generally greater than 0.9, indicating that the ACD calculation method is generally valid for the estimation of the partition coefficient. In a hydrolysis test, 4-NPI was reported to be hydrolytically unstable (see hydrolysis reaction below). Specifically, the hydrolysis half-lives of 4-NPI at 21 °C, in solvents consisting of aqueous buffers plus 1.8 vol. % CH_3CN, were 54 h, 6.4 h, and less than 0.5 h at pH 5, 7, and 9 respectively. The OECD partition coefficient test requires the equilibration of mixtures of 4-NPI, octanol, and water, which requires approx. 30 min, followed by the analyses of the octanol and aqueous phases, which would require an additional approx. 6 h, for a total test time of approx 6.5 h. This test duration is similar to the pH 7 half-life quoted above, and it follows that the extent of hydrolysis of 4-NPI during the P_{ow} test would be significant, and that this would have a significant affect the P_{ow} test result. Therefore, it was concluded that it is not possible to perform the partition coefficient test because of likely interference caused by the hydrolysis of 4-NPI during the test procedure. The calculated log (P_{ow}) values of 4-NPI and its hydrolysis product (4-NPI-H) were 1.5 ± 0.3 and 0.2 ± 0.4, respectively.



Reference: Reimer, G.J. (2002). Unpublished report no. 11291 1419 entitled "Physical/chemical property of 4-Nitro-N-Methylphthalimide [CAS RN 41663-84-7]: Partition Coefficient (OECD 107): Expert Statement", dated March 16, 2002 for General Electric Company, Pittsfield, MA, USA; from Reimer Analytical & Associates, Inc., Vancouver, BC, Canada.

Reliability: (Klimisch Code 1) Valid without restrictions.

5.0 WATER SOLUBILITY

5.1. SOLUBILITY

Value: 360 mg/L

Temperature: 25 °C

Description: Miscible [ ]; Of very high solubility [ ]; Of high solubility [ ]; Soluble [ ]; Slightly soluble [ ]; Of low solubility [X]; Of very low solubility [ ]; Not soluble [ ]

Method: OECD Test Guideline 105 (1993)

GLP: Yes [X] No [ ] ? [ ]

Test Substance: 4-Nitro-N-Methylphthalimide (4-NPI, CAS RN 41663-84-7); Lot UI0044 from General Electric Company; Purity: 96.4% 4-NPI, 3.6% 3-NPI received as a water slurry (~ 60% 4-NPI/40% water).

Remarks: It was not possible to perform the water solubility test on 4-NPI because of its likely hydrolysis during the test procedure. The calculated water solubilities of 4-NPI and its hydrolysis product (4-NPI-H) were 0.36 g/L and 1000 g/L respectively, at 25 °C and pH 7 (see above Partition Coefficient section 4.0 for a diagram of 4-NPI and it's hydrolysis product, 4-NPI-H). Water solubility was calculated using computer software manufactured by ACD (Advanced Chemistry Development, Inc., Toronto, Canada). Comparison of ACD-calculated water solubilities with experimentally observed values, for several different classes of organic compounds, showed correlations generally greater than 0.9, indicating that the ACD calculation method is generally valid for the estimation of water solubility. In a hydrolysis test, 4-NPI was reported to be hydrolytically unstable. Specifically, the hydrolysis half-lives of 4-NPI at

	21 °C, in solvents consisting of aqueous buffers plus 1.8 vol. % CH_3CN, were 54 h, 6.4 h, and less than 0.5 h at pH 5, 7, and 9 respectively. The OECD water solubility test (OECD Guideline 105) requires that a saturated aqueous solution of the Test Substance be obtained. Saturation is reached when the Test Substance concentration in aqueous solution has stabilized, which can require mixing the Test Substance with water for up to 96 h. This dissolution process is longer than the observed half-lives quoted above, and it is likely that hydrolysis of 4-NPI would occur to the extent of one half-life or more during the dissolution process. Additional hydrolysis would likely occur during the sample analysis steps. Therefore, it was concluded that it is not possible to perform the water solubility test because of likely interference caused by the hydrolysis of 4-NPI during the test procedure.
Reference:	Reimer, G.J. (2002). Unpublished report no. 11291 1419 entitled "Physical/chemical property of 4-Nitro-N-Methylphthalimide [CAS RN 41663-84-7]: Water Solubility (OECD 105)", dated March 16, 2002 for General Electric Company, Pittsfield, MA, USA; from Reimer Analytical & Associates, Inc., Vancouver, BC, Canada.
Reliability:	(Klimisch Code 1) Valid without restrictions.

5.2. pH VALUE, pKa VALUE

No studies were found.

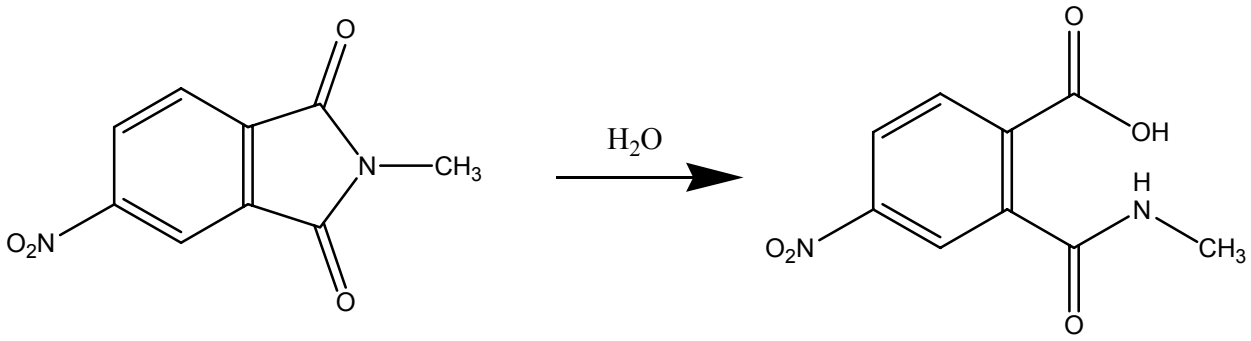
ENVIRONMENTAL FATE AND PATHWAYS

6.0 PHOTODEGRADATION

No studies were found.

7.0 STABILITY IN WATER

Type:	Abiotic (hydrolysis) [X]; biotic (sediment) [ ]
Half life:	54 h, 6.4 h, and less than 0.5 h at pH 5, pH 7, and pH 9 respectively.
Degradation:	Yes (See remarks below)
Method:	OECD Test Guideline 111 (1993)
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	4-Nitro-N-Methylphthalimide (4-NPI, CAS RN 41663-84-7); Lot UI0044 from General Electric Company; Purity: 96.4% 4-NPI, 3.6% 3-NPI received as a water slurry (~ 60% 4-NPI/40% water).
Remarks:	With respect to OECD Guideline 111, 4-NPI was hydrolytically unstable at pH 4. The hydrolysis of 4-NPI results in phthalimide ring opening to give the corresponding phthalamic acid hydrolysis product as shown below (Tirouflet, 1957; Flitsch, 1961).



pH 5 Buffer: An acetate buffer was prepared by dissolving 1.155 mL of glacial acetic acid in 1.0 L of deionized water solution (solution A; 0.02 M). Solution B was prepared by dissolving 1.64 g of anhydrous sodium acetate (or 2.72 g of the trihydrate) in 1.0 L of deionized water solution (0.02 M). Solution A (14.8 mL) and Solution B (35.2 mL) were mixed. The pH of this buffer was measured with a pH meter.
pH 7 Buffer: 3-N-morpholinopropanesulfonic acid (MOPS) free acid (20.93 g; 0.05 M) was dissolved in 2.0 L of deionized water solution. The solution was titrated to pH 7.0 with approximately 20 mL of 1.00 N aqueous NaOH.
pH 9 Buffer: Dissolved 1.222 g of ethanolamine (0.02 M) in approximately 900 ml of deionized water. The solution was titrated to pH 9.0 with approximately 32 mL of 1.0 M HCl, and made up to 1000 mL with deionized water.
4-NPI stock solution in CH_3CN (SkNPI2): 4-NPI (24.06 mg) was dissolved in 5.00 mL of CH_3CN solution in a volumetric flask (2.7508 mg/mL 4-NPI). After use the solution was stored in a Teflon-capped vial at -20 °C.

The calibration curve from the Calibration Solutions showed a linear relationship with a correlation coefficient (R^2) of 0.9999, indicating acceptable method precision for the analysis of 4-NPI in acetonitrile solution. The detection limit was defined as the lowest concentration of an analyte that an analytical process can reliably detect. The Instrument Detection Limit (IDL) was qualitatively estimated at 0.5 µg/mL (corresponding to 3 ng of 4-NPI injected on-column) for the detection of 4-NPI in acetonitrile solution, based on the chromatograms of Calibration Solutions.

Test Solutions of 4-NPI in solvents consisting of aqueous buffers plus 1.8 vol. % CH_3CN were placed in the injector tray of the HPLC instrument and were repeatedly injected over a 24 h period. During injections, the average air temperature at the vials was 21 ± 2 °C. The pH 5 and pH 7 chromatograms showed 4-NPI peaks at approximately 7.7 min, whereas no 4-NPI peaks were observed in the pH 9 Test Solution chromatograms, apparently because of complete hydrolysis of 4-NPI before the first HPLC injection of this Test Solution. Therefore, the half-life of 4-NPI at pH 9

was estimated at less than 0.5 h at 21 °C. The 4-NPI concentrations observed in the pH 5 and pH 7 Test Solutions indicate a higher rate of hydrolysis at pH 7 when compared to pH 5. 'First-order' kinetics were observed for the pH 7 hydrolysis of 4-NPI, with a corresponding half-life of 6.4 h. The pH 5 hydrolysis reaction, on the other hand, showed essentially 'zero-order' kinetics, and the corresponding half-life was 54 h.

Kinetics results for 4-NPI hydrolysis:

	21 ± 2 °C		
pH	**Rate Constant**	**Units**	**t1/2 (hr)**
5	6.82E-12	M sec^{-1}	54
7	3.00E-06	sec^{-1}	6.4
9	Not Determined	-	< 0.5

Reference: Reimer, G.J. (2002). Unpublished report no. SP11291 1419-Hy (BC Research Inc. Project no. 11291 1419) entitled "Physical/chemical property of 4-Nitro-N-Methylphthalimide [CAS RN 41663-84-7]: Hydrolytic Stability (OECD 111)", dated April 4, 2002 for General Electric Company, Pittsfield, MA, USA; from BC Research Inc., Vancouver, BC, Canada.

Iley, Jim; Calheiros, Teresa; Moreira, Rui, Phthalimidomethyl as a drug pro-moiety. Probing its reactivity. Bioorg. Med. Chem. Lett. (1998), 8(8), 955-958.

Su, S. C. K.; Shafer, J. A., Catalysis and inhibition of the hydrolysis of N-methylphthalimide by imidazole. J. Org. Chem. (1969), 34(4), 926-30.

Reliability: (Klimisch Code 1) Valid without restrictions.

8.0 TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS, INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION PATHWAYS

8.1 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

No studies were found.

9.0 BIODEGRADATION

No studies were found.

ECOTOXICOLOGICAL DATA

10.0 ACUTE/PROLONGED TOXICITY TO FISH

Type of Test:	Static [X] Semi-static [ ] Flow-through [ ] Other [ ] Open-system [ ] Closed-system [ ]
Species:	Rainbow trout (*Oncorhynchus mykiss)* (Rainbow Springs Hatchery, Thamesford, Ontario)
Exposure Period:	96 Hours
Results:	LC_{50} (96h) > 15.06 mg/L (nominal) NOEC = 15.06 mg/L (nominal) LOEC > 15.06 mg/L (nominal)
Analytical Monitoring:	Yes [X] No [ ] ? [ ]
Method:	OECD Test Guideline 203 (1993)
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	4-Nitro-N-Methylphthalimide (4-NPI, CAS RN 41663-84-7); Lot UI0044 from General Electric Company; Purity: 96.4% 4-NPI, 3.6% 3-NPI received as a water slurry (~ 60% 4-NPI/40% water).
Remarks:	Test conditions: Rainbow trout used for testing were obtained as eyed eggs from a certified disease-free hatchery. Eyed eggs were initially held in Heath Incubators. They were then transferred to fiberglass holding tanks of approximately 260 L capacity. Light was supplied by cool white fluorescent tubes on automatic timers which provide a 16-hour light / 8-hour dark photoperiod. Water temperature was maintained at a constant temperature of 15 ± 2°C. Rainbow trout were fed commercial No. 1 pellet size trout food at a daily ration approximating 1-4% wet body weight. Fish were weighed on a weekly basis to determine ration. Water quality necessary for the survival of the test organisms was continuously monitored and documented. Dilution water for rainbow trout culturing and testing was groundwater (average hardness ~ 290 mg/L as $CaCO_3$) from an aquifer in Aberfoyle, Ontario. Dilution water used for testing was adjusted to approximately pH 7.0, using hydrochloric acid. Laboratory dilution water was analyzed regularly for metals, organics, and inorganic chemicals. Samples (50 mL) for chemical analyses were collected from the control and all test concentrations used in the range-finding test at the beginning and end of the 96-hour test period. However, based on the results of the toxicological tests, only the control and the concentrations immediately bracketing the estimated 96-hour LC50 were sent for chemical verification. At the start and end of the definitive test, samples from each replicate in the control, lowest, middle and highest test solutions were pooled and a 50 mL sub-sample saved for analyses. All samples were frozen on dry ice in sealed 50 mL polypropylene centrifuge tubes before shipping to Reimer Analytical Associates, Inc. (Vancouver, B.C., Canada) for analysis. The test implemented was the static 96-hour acute lethality test using rainbow trout (*O. mykiss*) as the test organism. Fish were considered to be dead if there was no visible movement (e.g., gill movement), and if touching of the caudal peduncle produced no reaction (OECD, 1992). Chemical parameters (temperature, dissolved

oxygen, pH, and conductivity) were measured at each observation period (e.g., 24, 48, 72 and 96-hours). All stock solutions and exposure concentrations were dosed as product, but reported as nominal 4-NPI (active ingredient) concentrations (based on 100% product containing 50.2% 4-NPI). For the range-finding test, a 30 mg/L stock solution was prepared by mixing 0.5401 g of test product to 18 L of groundwater. For the definitive test, each replicate at each exposure concentration was prepared by adding an appropriate amount of test product to 15 L of groundwater, followed by thorough homogenization of each test concentration. Because of the rapid hydrolysis of the test product, each stock or exposure solution was stirred vigorously for approximately one hour before test initiation. The test substance was not readily soluble in water, and a large amount of fine, white, particulates were observed during preparation of the stock or exposure solutions during both the range-finding and definitive tests. Attempts were made to exclude most of the un-dissolved particles (thereby preventing possible ingestion by the test fish) by siphoning of the dissolved solution into new test containers. Definitive testing was conducted by setting up a dilution series that was based on the solubility of the test substance, and the results from a range-finding test. The dilution series was set up as a 2.2 geometric series to achieve five exposure concentrations (1.3, 2.8, 6.2, 13.6 and 30 mg/L; as test product) and a control. The definitive test was based on a total of 20 fish (i.e., 10 fish per replicate, in each of two replicates) exposed to each test concentration, as well as a control (100% dilution water). Mortality and abnormal behavior (e.g., erratic swimming) were recorded at 3, 24, 48, 72, and 96-hours, and any dead fish were removed. All fish were handled using a fine mesh dip net. An LC50 (concentration causing lethality to 50% of the organisms) was estimated from the 96-hour mortality data. The test was deemed valid if: i) mortality and impairment did not exceed 10% in the control, ii) constant conditions were maintained throughout the test, iii) the dissolved oxygen concentration was at least 60% of the air saturation value throughout the test, and iv) the concentration of the substance being tested was maintained (within 80% of nominal) throughout the test (if deviation from nominal was greater than 20%, results should be based on measured concentrations).

The following is a summary of test conditions:

Parameter	Test Condition
Test type	static
Duration	96 hours
Test organism / size	Juvenile rainbow trout (*Oncorhynchus mykiss*) 5 ± 1 cm long
Photoperiod	8-h dark and 16-h light
Light intensity	100 - 500 lux at the water surface
Temperature	15 ± 1 ºC
Dissolved oxygen	> 60% of saturation in control
Feeding	none during preceding 24 hrs and during test
Test vessel	15-L glass aquaria

Parameter	Test Condition
Test volume	15 L
Loading density	10 fish per test vessel (1.0 gram fish per liter)
Replicates	Two
Aeration	6.5 ± 1 mL/L/min (aerated to maintain at least 60% dissolved O_2)
Controls	100% dilution water
Nominal concentrations	0, 0.65, 3.11, and 15.06 mg/L (nominal 4-NPI as active ingredient)
Measured concentrations	0, 0, 0.005, and 2.51 mg/L
Criterion for effect	mortality and abnormal behavior (e.g., erratic swimming)
Calculated toxicity values	LC_{50} at 3, 24, 48, 72, and 96 h

Results: Samples of the control, low, medium and high exposure concentrations of the test product were analyzed for the active ingredient, 4-NPI, confirm the nominal test concentrations. The concentration of 4-NPI in the undiluted product was 50.3%. Recoveries of 4-NPI from spiked solutions were outside the acceptable 70 – 120 % range, presumably because of the rapid hydrolysis of 4-NPI. The difference between nominal and measured concentrations was greater than 20%. Similarly, the difference between old and new test solutions was greater than 20%. Based on these results, nominal 4-NPI concentrations (as active ingredient) were used in calculations of all test end points. With one exception, the toxicity test met all validity criteria. The concentration of the substance was not maintained within 80% of nominal, and measured concentrations were not used in endpoint calculations due the rapid hydrolysis of the test product. No fish died or exhibited abnormal behavior in any of the treated groups throughout the experiment. The 96-hour LC50 was determined to be greater than 15.06 mg/L (nominal 4-NPI as active ingredient).

Reference: Novak, L. (2002). Unpublished report no. S2184-02 entitled "Ecotoxicological evaluation of 4-Nitro-N-Methylphthalimide (CAS # 41663-84-7): Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*)", dated December, 2002, for General Electric Company, Pittsfield, MA, USA; from ESG International, Inc., Guelph, Ontario, Canada.

Reliability: (Klimisch Code 1) Valid without restrictions.

11.0 TOXICITY TO AQUATIC PLANTS (E.G. ALGAE)

Species: Green algae (*Selenastrum capricornutum*); ESG Ecotoxicity Laboratory (Guelph, ON).

End-point: Biomass [ ] Growth rate [X] Other [ ]

Exposure Period: 96 hours

Results: Growth:

EC_{50} (96h) = > 15.06 mg/L

NOEC = 4.65 mg/L

LOEC = 8.37 mg/L

Analytical Monitoring: Yes [X] No [ ] ? [ ]

Method: OECD Test Guideline 201 (1993)

GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	4-Nitro-N-Methylphthalimide (4-NPI, CAS RN 41663-84-7); Lot UI0044 from General Electric Company; Purity: 96.4% 4-NPI, 3.6% 3-NPI received as a water slurry (~ 60% 4-NPI/40% water).
Remarks:	Test conditions: The test organism, *S. capricornutum*, was cultured at the ESG Ecotoxicity Laboratory since August 1999. Cultures were aseptically transferred twice weekly and maintained in temperature and light controlled environments isolated from all testing facilities. The axenic nature of the stock culture was verified by plating on Trypticase Soy Agar (TSA) and Plate Count Agar (PCA). Algal growth curves were conducted semi-annually to ensure that algae are in an exponential growth phase and are suitable for testing. Samples (50 mL) for chemical analyses were collected from the control and all test concentrations used in the range-finding test at the beginning and end of the 96-hour test period. However, based on the results of the toxicological tests, only the control and the concentrations bracketing the estimated EC50 were analyzed. During the definitive test, 50-mL samples were collected from the control, lowest, two middle and highest test concentrations at 0 and 6 hours. At 96-hours only, the 50-mL sample was a sub sample of the pooled replicates from the control, lowest, two middle and highest test concentrations. For all collection periods, half of the samples were acidified to a pH of approximately 4 by adding 14 µL of 1N HCl. All samples were frozen and shipped on dry ice in sealed 50-mL polypropylene centrifuge tubes to Reimer Analytical & Associates, Inc. (Vancouver, BC, Canada) for analysis. Method verification included one control solution and one fortification of 3.88 mg/L. Only one spiking level was used because to the rapid hydrolysis of 4-NPI. The verification results were deemed acceptable if recoveries fell within the 70 - 120 % range. Following analysis of the verification samples, analyses of toxicity testing samples was performed. The samples were analyzed using high performance liquid chromatography (HPLC) to determine the exposure concentrations of 4-NPI. Analyses included the lowest concentrations that could be quantified, multi-point calibration curve, example chromatograms, method precision provided by the fortification sample, and a recovery study. All stock solutions and exposure concentrations were dosed as product, but reported as 4-NPI (active ingredient) concentrations. For the definitive test, a stock solution of approximately 30 mg product/L was prepared by mixing 30.3 mg of test product with 1000 mL of nutrient solution. The solution was then placed on a magnetic stir plate and agitated for 1 hour. Because of the rapid hydrolysis of the test product, all stock solutions were prepared approximately one-hour in advance of test initiation. The test substance was not readily soluble in water and some undissolved particulates were observed in the stock solutions during both the range and definitive tests. Definitive testing was conducted by setting up a dilution series to bracket the estimated EC50 calculated in a range-finding

test. The definitive multi-concentration EC50 test was conducted with nominal concentrations of 30.0, 16.67, 9.26, 5.14, 2.86 and 1.59 mg/L (a 1.8 dilution series, as test product), plus controls. Each concentration was replicated 4 times. Observations of cell number and appearance were conducted, using a hemocytometer, on a daily basis. After 96 hours of growth, the pH was measured in pooled samples from each concentration. Changes in cell development or appearance, such as cell clumping, cell morphology, cell color, cell shape, cell size, etc. were reported.

The following is a summary of the test conditions:

Parameter	Conditions
Test species	*Selenastrum capricornutum*
Duration of test	96 hours
Culture medium	Algal growth medium; axenically subcultured into fresh medium twice a week
Testing medium	Algal growth medium; filter sterilized, but otherwise treated in a non-axenic manner
Temperature	24 ± 1°C
Photoperiod	Continuous
Light intensity	Measured at the surface of the liquid in the flasks. 4 ± 10% kLux for culturing; 8 ± 20% kLux for testing
Test vessel	Clear glass 250-mL Erlenmeyer flasks covered with Jaece® non-toxic foam plugs
Nutrient/test solution volume	50 mL
pH of the test solutions	Measured, but not adjusted
Age of test plants	3-7 days at test initiation
Number of cells per test vessel	1×10^4 cells/mL
Range-finding test concentrations	0, 0.0015, 0.0151, 0.1506, 1.506, and 15.06 mg/L as active ingredient
Definitive test concentrations	0, 0.80, 1.44, 2.58, 4.65, 8.39 and 15.06 mg/L as active ingredient
Measured concentrations	Because of the rapid hydrolysis of 4-NPI, the percent recovery (measured/nominal) was < 80%.
Number of replicate test vessels/concentration in definitive test	4 treatment and control replicates
Measured water quality parameters	pH at start and end of the test in all concentrations
Measured endpoints	Cell number measured daily using a hemocytometer
Calculated endpoints	Area under the growth curve, growth rate, cell number
Test validity criteria	1.6×10^5 cells/mL after 72 hours (OECD, 1984)

Results: For the definitive test, samples of the control, low, two medium and high exposure concentrations of the test product were analyzed for the active ingredient, 4-NPI, to confirm the nominal test concentrations. Samples were collected at 0, 6 and 96 hours and half of these were acidified, while the other half was not acidified. The concentration of 4-

	NPI in the undiluted product was 50.2%. Recoveries of 4-NPI from spiked solutions were outside the acceptable 70 – 120 % range, presumably because of the rapid hydrolysis of 4-NPI. The percent recovery (measured/nominal) was less than 80%. The difference between 0- and 96-hour test solutions was greater than 20%. The acidification reduced the rate of hydrolysis, but did not eliminate it. Nominal 4-NPI concentrations were used in calculations of all test end points. Based on 4-NPI active ingredient nominal concentrations during the definitive test, the 72- and 96-hour EC50s for cell number were 5.54 and 10.52 mg/L, respectively. The 72- and 96-hour EC50s for area under the growth curve were 5.36 and 7.80 mg/L, respectively. The 0 to 72 and 0 to 96-hour EC50s for growth rate were > 15.06 mg/L, the highest concentration of active ingredient tested. For four endpoints (72-hour cell number, 72- and 96-hour area under the growth curve and 72 hour growth rate), the NOECs were 1.44 mg/L and the LOECs were 2.58 mg/L. For 96-hour cell number, the NOEC and LOEC were 2.58 and 4.65 mg/L, respectively. For 96-hour growth rate, the NOEC and LOEC were 4.65 and 8.37 mg/L, respectively.
Reference:	Roshon, R. (2002). Unpublished report no. S2184-01 entitled "4-Nitro-N-Methylphthalimide (4-NPI; CAS # 41663-84-7): Growth Inhibition Test with the Freshwater Green Alga, *Selenastrum capricornutum*, (OECD 201)", dated December, 2002, for General Electric Company, Pittsfield, MA, USA; from ESG International, Inc., Guelph, Ontario, Canada.
Reliability:	(Klimisch Code 1) Valid without restrictions.

12.0 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

12.1.1 Daphnia

Type of Test:	Static [X] Semi-static [ ] Flow-through [ ] Other [ ] Open-system [ ] Closed-system [ ]
Species:	*Daphnia magna*
Exposure Period:	24 Hours
Results:	EC_{50} (24h) > 15.06 mg/L NOEC > 15.06 mg/L
Analytical Monitoring:	Yes [X] No [ ] ? [ ]
Method:	OECD Test Guideline 202 (1993)
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	4-Nitro-N-Methylphthalimide (4-NPI, CAS RN 41663-84-7); Lot UI0044 from General Electric Company; Purity: 96.4% 4-NPI, 3.6% 3-NPI received as a water slurry (~ 60% 4-NPI/40% water).
Remarks:	Test conditions: *D. magna* STRAUS were cultured continuously from a disease-free population that has been actively reproducing in ESG's laboratory for the past eleven years. The organisms were originally obtained from the Ontario Ministry of the Environment (OMOE; Rexdale, Ontario, Canada). Periodic taxonomic confirmation of representative organisms from the population is conducted to verify the taxonomy of *D. magna*. Cultures were maintained in 1L polystyrene containers

containing 800 mL of groundwater (dilution water). Two to four week old adult daphnids were transferred to fresh water daily, and fed a 3:1 mixture of *Selenastrum capricornutum* and *Chlorella fusca* (green algae) at a rate of 10 mL/L (density of 3.5 X 10^7 cells/mL). The algal diet was supplemented with a suspension of yeast, Cerophyll, and commercial trout pellets, fed daily at a rate of 5 mL/L. Culture temperature and photoperiod were identical to that of the test system (temperature 20 ± 1ºC, and a photoperiod of 16 hours light and 8 hours of darkness with a 30-minute transition period). Neonate test organisms were obtained from the stock cultures following the daily transfer of adult daphnids, therefore ensuring that test organisms were of a uniform age (i.e. < 24h old). Water quality necessary for the survival of the test organisms was continuously monitored and documented. Dilution water for *D. magna* culturing and testing was moderately hard groundwater from an aquifer in Aberfoyle, Ontario, Canada that is continuously and vigorously aerated. Dilution water used for testing was adjusted to approximately pH 7.0, using hydrochloric acid. Laboratory dilution water is analyzed regularly for metals, organics, and inorganic chemicals.

Samples (50 mL) for chemical analyses were collected from the control and all test concentrations used in the range-finding test at the beginning and end of the 24-hour test period. However, based on the results of the toxicological tests, only the control and the concentrations immediately bracketing the estimated EC50 were sent for chemical verification. At the start and end of the definitive test, samples from each replicate in the control, lowest, middle and highest test solutions were pooled and a 50 mL sub-sample saved for analyses. All samples were frozen before shipping to Reimer Analytical & Associates, Inc. (Vancouver, BC, Canada) on dry ice in sealed 50 mL polypropylene centrifuge tubes for analysis. Method verification was performed, which included one control solution and one fortification of 3.88 mg/L. Only one spiking level was used because to the rapid hydrolysis of 4-NPI. The verification results were deemed acceptable if recoveries fell within the 70 - 120 % range. This range is centered at less than 100 % because it applies to a method, which involves an extraction step, where a recovery of less than 100 % is expected. Following analysis of the verification samples, analyses of toxicity testing samples was performed. The samples were analyzed using high performance liquid chromatography (HPLC) to determine the exposure concentrations of 4-NPI. Analyses included the lowest concentrations that could be quantified, multi-point calibration curve, example chromatograms, method precision provided by the fortification sample, as well as a recovery study.

All stock solutions and exposure concentrations were dosed as product, but reported as nominal 4-NPI (active ingredient) concentrations (based on 100% product containing 50.2% 4-NPI). For the range-finding test, a 30 mg/L stock solution was prepared by mixing 0.0302 g of test product to 1 L of groundwater. For the definitive test, a 30 mg/L stock solution was prepared by mixing 0.0301 g of test product to 1 L of groundwater. Because of the rapid hydrolysis of the test product, all stock solutions

were prepared approximately one hour in advance of test initiation. The test substance was not readily soluble in water, and a large amount of fine, white, particulates were observed in the stock solutions during both the range-finding and definitive tests.

The test implemented was the static 24-h acute immobilization test using *D. magna* (neonates, #24 h old) as the test organism. Test organisms were considered to be immobile if they were unable to swim within 15 seconds following gentle agitation of the test solution. Chemical parameters (dissolved oxygen, pH, and conductivity) were measured in initial and final samples. Dilution water hardness was measured at the beginning of the test.

Definitive testing was conducted by setting up a dilution series that was based on the solubility of the test substance, and the results from the range-finding test. The dilution series was set up as a 2.2 geometric series to achieve five exposure concentrations (1.3, 2.8, 6.2, 13.6 and 30 mg/L; as test product) and a control. The definitive test was based on a total of 20 daphnids (i.e., five (5) daphnids per replicate, in each of four (4) replicates) exposed to each test concentration, as well as a control (100% dilution water). Immobility and abnormal behavior (e.g., erratic swimming) was recorded at 24 hours. An EC50 (concentration causing immobility in 50% of the organisms) was estimated from the 24-hour immobility data. The test was considered valid if: i) immobility did not exceed 10% in the control, and ii) dissolved oxygen was greater than or equal to 60 % of the air saturation value at test completion.

The following is a summary of test conditions:

Parameter	Test Condition
Test type	Static
Duration of test	24 hours
Test organism	*Daphnia magna*.
Temperature	20 $\pm$ 1 ºC
Photoperiod	16-hour light, 8-hour dark (with 30 minute transition period)
Light intensity	400 - 800 lux at the water surface
Feeding	None (during preceding 24 h and during testing)
Test vessel	250 mL glass beakers
Test volume	100 mL minimum
Replicates	4 treatment and control replicates
Aeration	None
Controls	Groundwater (initial hardness approximately 200 mg/L as $CaCO_3$; initial pH approximately 8.3) adjusted to approximately pH 7 before testing.
Nominal concentrations	0, 0.65, 3.11, and 15.06 mg/L as active ingredient
Measured concentrations	0, 0, 0.012, and 0.202 mg/L
Criterion for effect	Immobilization
Calculated toxicity values	EC_{50} at 24h

Results: Samples of the control, low, medium and high exposure concentrations of the test product were analyzed for the active ingredient, 4-NPI, in order to confirm the nominal test concentrations. The concentration of 4-NPI in the undiluted product was 50.2%. Recoveries of 4-NPI from spiked solutions were outside the acceptable 70 – 120 % range, presumably because of the rapid hydrolysis of 4-NPI. Because of the rapid hydrolysis of 4-NPI, the difference between nominal and measured concentrations was greater than 20%. Similarly, the difference between old and new test solutions was greater than 20%. Based on these results, nominal 4-NPI concentrations (as active ingredient) were used in calculations of all test end points.
The test met all validity criteria. Immobile organisms were not observed at any exposure concentration. Therefore, the 24-hour EC50 was determined to be greater than 15.06 mg/L (nominal 4-NPI as active ingredient).

Reference: Novak, L. (2002). Unpublished report no. S2184-03 entitled "Ecotoxicological evaluation of 4-Nitro-N-Methylphthalimide (CAS # 41663-84-7): Acute Toxicity to *Daphnia magna*", dated December, 2002, for General Electric Company, Pittsfield, MA, USA; from ESG International, Inc., Guelph, Ontario, Canada.

Reliability: (Klimisch Code 1) Valid without restrictions.

TOXICITY

13.0 ACUTE TOXICITY

13.1 ACUTE ORAL TOXICITY

Type: LD_0 [ ]; LD_{100} [ ]; LD_{50} [X]; LDL_0 [ ]; Other [ ]
Species/strain: Rat/Sprague-Dawley Crl:CD®BR
Sex: Males and females
animals: 5/sex
Vehicle: 0.5% Carboxymethyl cellulose
Value: 2.80 g/kg (95% confidence interval: 2.22-3.53 g/kg)
Method (Year): Other. Comparable to OECD 401 test guideline.
Before test compound administration, young adult rats (supplied by Charles River Breeding Laboratories, Portage, MI) weighing between 200 and 300 grams were housed in groups of 4 to 6, separated by sex, in metal wire mesh colony cages. Food and water were available *ad libitum*, except food was withheld for 16-18 hours before and approximately 5 hours after dosing. The rats used in the study were acclimated for approximately one week before study initiation. Groups of 10 rats (5/sex) were then dosed by oral gavage with 2.0, 3.5, or 5.0 g/kg 4-NPI, suspended in 0.5% methylcellulose at a volume $\leq$ 20 ml/kg. Following dosing, the animals were housed individually and observed for signs of toxicity frequently during a 3.5 hour period immediately following dosing and twice daily thereafter for 14 days. Body weights were recorded on the day of dosing

and twice weekly thereafter for 14 days. Gross necropsy was performed on all rats at the time of death or at the end of the 14 day observation period. The LD50 value (with 95% confidence levels) was determined using the method of Litchfield and Wilcoxin.

GLP: Yes [X] No [ ] ? [ ]

Test substance: UI80-2 [4-NPI (CAS# 41663-84-7) Supplied by General Electric Plastics, Mt. Vernon, IN]
Commercial, purity: Not specified but typically > 95% 4-NPI (<5% 3-NPI)

Remarks: In the 5.0 g/kg group, all rats were moderately to severely hypoactive by 30 minutes after dosing and had pale blue extremities. Within 24 hours, 9 of 10 rats were found dead. The one surviving animal exhibited hypoactivity and dyspnea until its death on day 3. In the 3.5 g/kg group, all rats were hypoactive within 30 minutes. After 90 minutes, 3 rats were found dead and the survivors had pale blue extremities. By 24 hours 4 additional rats died. The remaining animals remained hypoactive for up to 7 days, with one found dead on day 6. In the 2.0 g/kg group, all rats were slightly hypoactive within 90 minutes until 24 hours. Two rats remained hypoactive for 3 days and one was found dead on day 3. The total number of animals found dead per group is listed below:

Dose (g/kg)	Mortality Data
2.0	1/10
3.5	8/10
5.0	10/10

Gross necropsy at the time of death following the 14 day observation period revealed yellow and/or red fluid in the stomach and intestines in 18 of 30 rats, gas filled and distended stomachs in 3 of 30, mottled and pale and/or lobularly accentuated livers in 4 of 30, darkened spleens in 9 of 30 and darkened kidneys in 2 of 30 rats. All tissue and organ changes were considered to be treatment-related.

Reference: Rosenberger, S. M. Unpublished report no. 100A-101-010-80 entitled "Evaluation of UI80-2 for acute oral toxicity in rats, primary dermal and ocular irritation in rabbits, and acute dermal toxicity in rabbits" dated June 11, 1981 from Toxicology and Pathology Services (TPS), Mt. Vernon, IN for General Electric Plastics, Mt. Vernon, IN

Reliability: (Klimisch Code 1) Valid without restrictions

13.2 ACUTE INHALATION TOXICITY

Type: LD_0 [ ]; LD_{100} [ ]; LD_{50} [X]; LDL_0 [ ]; Other [ ]

Species/strain: Rat/Sprague-Dawley Crl:CD®BR

Sex: Males and females

animals: 5/sex

Value: > 36.0 mg/L

Method (Year): Other. Comparable to OECD 403 test guideline.
Groups of rats (5/sex), weighing 244 to 277 grams, were placed in a sealed 59.1 L glass chamber and exposed to an atmospheric concentration of 36.0

	mg/L of 4-NPI for 4 hours. Observations for clinical signs of toxicity and mortality were made during the exposure and daily thereafter for 14 days.
GLP:	Yes [ ] No [X] ? [ ]
Test substance:	AR 85993 [4-NPI (CAS# 41663-84-7) Supplied by General Electric Plastics, Mt. Vernon, IN] Commercial, purity: Not specified but typically > 95% 4-NPI (<5% 3-NPI)
Remarks:	None of the rats exposed to the atmospheric concentration of 36.0 mg/L died during the 4 hour exposure period or the subsequent 14 day observation period. Clinical signs of toxicity observed during the exposure period included eye squint, dyspnea, salivation, nasal porphyrin discharge, erythema, and decreased activity. At 24 hours, all rats were normal and remained so for the duration of the study period. All rats exhibited normal body weight gains during the study period.
Reference:	Dean, W. P. Unpublished report no. 313-087 entitled "Acute inhalation toxicity in albino rats" dated June 8, 1976 from International Research and Development Corporation, Mattawan, MI for General Electric Plastics, Mt. Vernon, IN
Reliability:	(Klimisch Code 2) Valid with restrictions. An acceptable non-GLP study with basic data provided. No analytical or particle size information provided.

13.3 ACUTE DERMAL TOXICITY

Type:	LD_0 [ ]; LD_{100} [ ]; LD_{50} [X]; LDL_0 [ ]; Other [ ]
Species/strain:	Rabbit/New Zealand White
Sex:	Males and females
# animals:	5/sex
Vehicle:	0.9% Saline solution
Value:	> 2 g/kg
Method (Year):	Other. Comparable to OECD 402 test guideline. Before test compound administration, young adult rabbits (supplied by Kuiper Rabbit Ranch, Gary, IN) weighing between 2.20 and 3.20 kg each were housed individually in metal hanging cages. Food and water were available *ad libitum*. The rabbits used in the study were acclimated for 18 days before study initiation. Their dorsal and dorsolateral trunk, from which hair was clipped closely 24 hours before dosing, was free of pre-existing skin lesions. A 2 g/kg dose of 4-NPI was moistened with saline (0.9%)and applied topically to the clipped back of a group of 10 rabbits (5/sex). A tongue depressor was used for spreading the test material over an area of approximately 10% of the body surface. All rabbits had the skin abraded with a sterile hypodermic needle before application. Abrasions were of sufficient depth to penetrate the stratum corneum, but not deep enough to disturb the underlying dermis or induce bleeding. Following the application, the treated area of skin was covered with rubber dental dam and secured with rubberized clothe. Rabbits were then returned to their cages, the wraps were removed after 24-hours, the volume of unabsorbed material was estimated and the backs were rinsed with lukewarm water and patted dry. The animals were observed twice daily for toxic effects for 14 days. Individual body weights and food consumption were recorded twice

	weekly. Gross necropsy was performed on all rabbits at termination of the study and treated and untreated skin sections as well as any remarkable organs or tissues were preserved in 10% formalin for histopathological examination.
GLP:	Yes [X] No [ ] ? [ ]
Test substance:	UI80-2 [4-NPI (CAS# 41663-84-7) Supplied by General Electric Plastics, Mt. Vernon, IN] Commercial, purity: Not specified but typically > 95% 4-NPI (<5% 3-NPI)
Remarks:	Following 24 hours after application, all 0.9% saline had been absorbed and there appeared to be 100% unabsorbed 4-NPI at the test sites. All dermal test sites appeared unremarkable and no signs of systemic toxicity or death were noted for any of the animals during the 14-day observation period. Body weight loss was observed in 4 of 5 females and in 1 of 5 males on day 14. Food consumption was decreased in both males and females during the first 7 days after treatment. Gross necropsy and subsequent microscopic examination at the end of the 14 day observation period did not reveal any consistent or distinct treatment-related tissue or organ change.
Reference:	Rosenberger, S. M. Unpublished report no. 100D-303-210-80 entitled "Evaluation of UI80-2 for acute oral toxicity in rats, primary dermal and ocular irritation in rabbits, and acute dermal toxicity in rabbits" dated June 11, 1981 from Toxicology and Pathology Services (TPS), Mt. Vernon, IN for General Electric Plastics, Mt. Vernon, IN
Reliability:	(Klimisch Code 1) Valid without restrictions

14.0 GENETIC TOXICITY *IN VITRO* OR *IN VIVO* (CHROMOSOMAL ABERRATIONS)

Type:	*In vitro* cytogenetic assay			
System of testing:	Chinese hamster ovary (CHO) cells			
Concentration:	Up to 900 μg/mL			
Metabolic activation:	With [ ]; Without [ ]; With and Without [X]; No data [ ]			
Results:				
Cytotoxicity conc:	With metabolic activation:	500 μg/mL		
	Without metabolic activation:	500 μg/mL		
Precipitation conc:	> 500 μg/mL			
Genotoxic effects:		+	?	-
	With metabolic activation:	[ ]	[ ]	[X]
	Without metabolic activation:	[ ]	[ ]	[X]
Method (Year):	Other (1981). The protocol is comparable to OECD Test Guideline 471 *Description of test procedure*: Cultures were exposed to 4-NPI in a series of test concentrations, with the highest dose tested at 1000 μg/mL. Before fixation, cultures were examined for degree of confluence and presence of large, rounded (mitotic cells). Only those flasks expected to yield at least some dividing cells were fixed. In the assay without metabolic activation, one day after culture initiation, approximately 3 x 10^6 cells were treated with the test article for 8 ½ to 10 hours. The cultures			

were then washed with saline and fresh culture medium was added, with colcemid at a final concentration of 0.1 μg/mL. Approximately 2 hours later, metaphase cells were collected by mitotic shake-off. The cells were swollen with 0.075 M KCl hypotonic solution, then washed 3 times in fixative (methanol:acetic acid, 3:1), dropped onto slides and air dried. In the activation system, cells were incubated for 2 hours in the presence of 4-NPI and the S9 reaction mixture, in growth medium without fetal calf serum. After the 2 hour exposure period, cells were washed at least twice with buffered saline and normal growth medium containing 10% fetal calf serum was added. Incubation was continued for another 8-10 hours with colcemid present during the last 2 hours. Thereafter, the procedure was the same as described above. Slides were stained with 5% Giemsa at pH 6.8 for scoring of chromosome aberration frequencies. One hundred cells were scored per dose. Chromatid and chromosome gaps, breaks, fragments, and reunion figures, as well as numerical aberrations such as polyploid cells were scored.

GLP: Yes [X] No [ ] ? [ ]

Test substance: UI81-1 [4-NPI (CAS# 41663-84-7) Supplied by General Electric Plastics, Mt. Vernon, IN]
Commercial, purity: Not specified but typically > 95% 4-NPI (<5% 3-NPI)

Remarks: In the tests without metabolic activation, the aberration frequencies in the negative and solvent control cultures in trial 1 were in the normal historical range for the laboratory (0.0 and 2.0% cells with aberrations), respectively). The aberration data for cultures exposed to 4-NPI in trial 1 showed a slight upward trend (4.0, 0.0, 6.0, 6.0, and 9.0% for the 300, 350, 400, 450, and 500 μg/mL groups, respectively), but were not statistically significant. In trial 2, the negative and solvent controls were within the normal range (2.0 and 5.0%) and no increase in aberrations were found in cells exposed to 4-NPI (3.0, 3.0, and 4.0% in the 339.8, 388.3, and 485.4 μg/mL groups, respectively). In the trial 3, five cultures were exposed to doses ranging from 600-1000 μg/mL and white precipitate was observed in all cultures. There was no increase in cells with aberrations (2.0-4.0%), thus the very slight increase seen in trial 1 was not reproducible. The positive control, Mitomycin C, produced a significant increase in cells with aberrations in all 3 trials (20, 25, and 23%).
In the test with metabolic activation, trial 1 was abandoned because of microbial contamination. In trial 2, the aberration data for the negative and solvent controls were within historical range and no increase in aberrations were found in cells exposed to 4-NPI (3.0, 4.0, 2.0, 1.0, and 2.0% in the 600, 700, 800, 900, and 1000 mg/mL groups, respectively). The positive control, cyclophosphamide, produced a significant increase in cells with aberrations (37.0%).
In conclusion, 4-NPI did not cause any meaningful increase in chromosome damage and was considered to be negative in the test under the conditions of the assay.
Criteria for evaluating results: If the test article or its metabolites produce a statistical change (Student t-test) in the estimated number of breaks involved in production of different types of aberrations or the frequency of

cells with more than one aberration, it was considered to be positive in the test. Also, any evidence for increasing amounts of damage with increasing dose (positive dose trends) was also factored in the determination for mutagenic potential.
Plates/test: Single plates per test concentration, but tested in three separate trials (triplicate)
Activation system: The S-9 fraction from rat (adult male Sprague-Dawley) liver induced with Aroclor 1254.
Media: CHO cells for this assay were grown in McCoy's 5a medium supplemented with 10% fetal calf serum, L-glutamine, and antibiotics. Cultures were set up 24 hours before treatment by seeding 8 x 10^5 cells per 75 cm^2 plastic flask in 10 mL of fresh medium.

Reference: Galloway, S.M 1981. Unpublished report 20990 entitled "Mutagenicity evaluation of UI 81-1 in an *in vitro* cytogenetic assay measuring chromosome aberration frequencies in Chinese hamster ovary (CHO) cells" dated November 17, 1981 from Litton Bionetics, Inc., Kensington, MD, for General Electric Plastics, Mt. Vernon, IN

Reliability: (Klimisch Code 1) Valid without restrictions.

15.0 GENETIC TOXICITY *IN VITRO*

15.1 BACTERIAL TEST

Type: Reverse mutation assay

System of testing: Bacteria (*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538

Concentration: 0, 1, 10, 100, 500, 1000, 2500, 5000, and 10000 μg/plate

Metabolic activation: With [ ]; Without [ ]; With and Without [X];
No data [ ]

Results:

Cytotoxicity conc.: With metabolic activation: ≥ 5000 μg/plate
Without metabolic activation: ≥ 5000 μg/plate

Precipitation conc.: 10000 μg/plate

Genotoxic effects:

	+	?	-
With metabolic activation:	[X]	[ ]	[ ]
Without metabolic activation:	[X]	[ ]	[ ]

Method (Year): Based on Ames et al. (1975) *Mut. Res*., 31:347. The protocol is comparable to OECD Test Guideline 471
Description of test procedure: Doses used in the mutagenicity assays were selected from a preliminary toxicity test performed on the strain TA-100. Fourteen doses ranging from 1.0 μg to 10,000 μg per plate were used. In the mutagenicity assays, at least six doses were used with the highest dose exhibiting a 50% toxicity. For the mutagenicity testing (without metabolic activation) the following is added to a sterile test tube placed in a water bath (43-45 C): 2.00 mL of 0.6% agar containing 0.05 mM histadine and 0.05 mM biotin, 0.05 mL of a solution of the test

material to give the appropriate dose, 0.1 to 0.2 mL of indicator organisms, and 0.5 mL of 0.2M phosphate buffer (pH 7.4). This mixture is swirled gently and then poured on minimal agar plates and incubated at 37 C for approximately 2 days. In the activation system, the same procedure is following with the exception of the addition of 0.5 mL of S9 mix to the tubes in place of 0.5 mL of phosphate buffer. A negative control consisting of the solvent (DMSO) used for the test material was also assayed concurrently with the test material. Specific positive controls known to interact with each strain were also used and assayed concurrently with the test material. In the non-activated system, sodium azide (10.0 µg/plate) was used for strains TA1535 and TA100, 2-nitrofluorene (10.0 µg/plate) was used for strains TA1538 and TA98, and 9-aminoacridine (50.0 µg/plate) was used for strain TA1537. In the S9 activated system, 2-anthramine (2.5 µg/plate) was used for all strains.

GLP: Yes [X] No [ ] ? [ ]

Test substance: UI81-2 [4-NPI (CAS# 41663-84-7) Supplied by General Electric Plastics, Mt. Vernon, IN]
Commercial, purity: Not specified but typically > 95% 4-NPI (<5% 3-NPI)

Remarks: The results of the tests conducted on the compound in the presence and absence of the metabolic activation system were positive with strains TA-1538, TA-98, and TA-100. The mutagenic effects were observed at a minimum concentration of 2500 µg/plate with TA98 and TA100 and at 10000 µg/plate with TA1538. All positive controls were mutagenic in their respective strains and activation systems.
Criteria for evaluating results: The procedures used to evaluate the mutagenicity of the test material were semiquantitative. The criteria used to determine positive effects were based primarily on historical data, the dose response pattern, reproducibility, and positive and negative control values within the experiment. For strains TA1535, TA1537, and TA1538, if the solvent control value was within normal range, a test material producing a positive response equal to three times the solvent control value was considered mutagenic. For strains TA98 and TA100, a test material producing a positive response equal to twice the solvent control value was considered mutagenic.
Plates/test: Samples were run in duplicate, with and without metabolic activation.
Activation system: The S-9 fraction from rat (adult male Sprague-Dawley) liver induced with Aroclor 1254.
Media: Aqueous agar solution (see above methods section).

Reference: Jagannath, D.R. 1981. Unpublished report 20988 entitled "Mutagenicity evaluation of UI 81-2 in the Ames *Salmonella*/microsome plate test" dated December 16, 1981 from Litton Bionetics, Inc., Kensington, MD, for General Electric Plastics, Mt. Vernon, IN

Reliability: (Klimisch Code 1) Valid without restrictions.

Additional References for Bacterial Genetic Toxicity Tests: Several other bacterial mutagenicity assays using the same *Salmonella typhimurium* strains and study design were also conducted for 4-NPI. In an earlier assay, 4-NPI

was not mutagenic, either with or without metabolic activation at concentrations up to 2000 µg/plate (Jagannath, 1977). However, the highest concentration of 2000 µg/plate was clearly not cytotoxic and did not satisfy the criteria for establishing a high concentration. In a third study, mutagenic activity was observed at a concentration of 10000 µg/plate with TA1538 in the activation assay and with TA98 in both the non-activation and activation assays (Jagannath, 1981). In a fourth study (Finch, 1981), 4-NPI tested positive in TA98 at concentrations of 2500 and 5000 µg/plate, in TA100 at 500 µg/plate and higher, and in TA1538 at 1000, 2500, and 5000 µg/plate in the presence of the S-9 activation system. In the absence of metabolic activation 4-NPI tested positive at all concentrations (50 µg/plate and higher) with TA98, and at concentrations of 500 µg/plate and higher in strains TA100 and TA1538.

Jagannath, D.R., 1977. Unpublished report 20838 entitled "Mutagenicity evaluation of 09-77-011154-018" dated November 22, 1977 from Litton Bionetics, Inc., Kensington, MD, for General Electric Plastics, Mt. Vernon, IN

Jagannath, D.R., 1981. Unpublished report 20988 entitled "Mutagenicity evaluation of 02-81-011535-012 AR #93478 in the Ames *Salmonella*/microsome plate test" dated May 27, 1981 from Litton Bionetics, Inc., Kensington, MD, for General Electric Plastics, Mt. Vernon, IN

Finch, R. A. 1981. Unpublished report 81562 entitled "Determination of the mutagenic potential of 4-Nitro-N-Methylphthalimide (4-NPI) using the Plate Incorporation Method of the Ames Salmonella/Microsome mutagenicity test" dated August 14, 1981 from Raltech Scientific Services, Madison, WI for Bofors Lakeway, Inc., Muskegon, MI

15.2 NON-BACTERIAL IN VITRO TEST (MAMMALIAN CELLS)

Type:	Mammalian cell gene mutation assay			
System of testing:	Mouse lymphoma cell line (L5178 TK+/-)			
Concentrations:	up to 1000 µg/mL without metabolic activation Up to 20 µg/mL with metabolic activation			
Metabolic activation:	With [ ]; Without [ ]; With and Without [X]; No data [ ]			
Results:				
Cytotoxicity conc.:	With metabolic activation:	> 20 µg/mL		
	Without metabolic activation:	> 1000 µg/mL		
Precipitation conc.:	> 1000 µg/mL			
Genotoxic effects:		+	?	-
	With metabolic activation:	[X]	[ ]	[ ]
	Without metabolic activation:	[ ]	[ ]	[X]
Method (Year):	Based on the publication by Clive and Spector (1975) *Mut. Res*., 31:17-29. The protocol is comparable to OECD Test Guideline 471 *Description of test procedure*: The solubility of the test chemical in growth medium and/or DMSO was first determined. A wide range of concentrations was tested for cytotoxicity, starting with the maximum applied dose of 10 mg/mL. After an exposure time of four hours, the			

	cells were washed and a viable cell count was obtained the following day. Relative cytotoxicities expressed as the reduction in growth compared to the growth of untreated cells were used to select up to ten doses that cover the range from 0 to 50-90% reduction in 24 hours of growth. These selected doses (62.5, 125, 250, 500, and 1000 μg/mL without metabolic activation and 0.977, 1.950, 3.910, 7.810, and 15.600 μg/mL with metabolic activation in trial 1 and 1.250, 2.500, 5.000, 10.000, 15.000, and 20.000 in trial 2) were applied to cell cultures prepared for mutagenicity testing. In the non-activation assay, cultures were exposed to 4-NPI for four hours and washed and placed in growth medium for two to three days to allow recovery, growth, and expression of the induced TK -/- phenotype. Cell counts were determined daily. At the end of the expression period, 3×10^6 cells for each selected dose were seeded in soft agar plates with selection medium and resistant (mutant) colonies were counted after 10 days of incubation. To determine the actual number of cells capable of forming colonies, a portion of the cell suspension was also cloned from normal medium. The ratio of resistant colonies to total viable cell number was the mutant frequency. For the S9 activation assay, the only difference from above was the addition of the S9 fraction of rat liver homogenate (50 μL) and necessary cofactors (2.4 mg NADP/mL and 4.5 mg isocitric acid/mL) during the four-hour treatment period. The S9 homogenate was prepared from Fischer 344 adult male rat liver induced by Aroclor 1254.
GLP:	Yes [X] No [ ] ? [ ]
Test substance:	UI81-1 [4-NPI (CAS# 41663-84-7) Supplied by General Electric Plastics, Mt. Vernon, IN] Commercial, purity: Not specified but typically > 95% 4-NPI (<5% 3-NPI)
Remarks:	In the non-activation assay, no test concentration exceeded the minimum criterion for mutagenesis (mutant frequency of 32.5×10^{-6}). The mutant frequencies were 14.3 and 11.1 x 10^{-6} for the negative solvent control and 18.6, 31.1, 17.4, 25.9, and 24.2 x 10^{-6} for the 62.5, 125, 250, 500, and 1000 μg/mL groups, respectively. The positive control (0.5 μL/mL ethylmethane sulfonate; EMS) had a significant mutant frequency of 771.1 x 10^{-6}. In the activation system, the minimum criterion for mutagenesis was a mutant frequency exceeding 78.7 x 10^{-6}. This value was exceeded in all 4-NPI test groups. The mutant frequencies were 53.2 and 39.3 x 10^{-6} for the negative solvent control and 163.2, 127.2, 139.1, 234.9, and 118.8 x 10^{-6} for the 0.977, 1.950, 3.910, 7.810, and 15.600 μg/mL groups, respectively. The positive control (0.3 μL/mL dimethylnitrosamine; DMN) had a significant mutant frequency of 182.6 x 10^{-6}. In trial 2 of the activation system, the 4-NPI again exceeded the minimum criteria for mutagenesis (46.9 x 10^{-6}) at all test concentrations. The mutant frequencies were 24.3 and 22.8 x 10^{-6} for the negative solvent control and 54.1, 56.9, 67.4, 73.0, 87.2, and 121.4 x 10^{-6} for the 1.250, 2.50, 5.00, 10.00, 15.00, and 20.00 μg/mL groups, respectively. The positive control had a significant mutant frequency of 336.1 x 10^{-6}. In conclusion, 4-NPI was non mutagenic without metabolic activation and positive for mutagenicity in the presence of S9 metabolic activation.

Criteria for evaluating results: The minimum condition determined necessary to demonstrate mutagenesis was a mutant frequency that exceeded 150% of the concurrent backgroup frequency by at least 10×10^{-6}. The background frequency is the average mutant frequency of the solvent and untreated controls. For this assay in the non-activation system, the minimum criterion was a mutant frequency that exceeded 32.5×10^{-6}. In the activation assay, a mutant frequency of 78.7×10^{-6} was required in trial 1 and a mutant frequency of 46.9×10^{-6} in trial 2. Also, a dose-related or toxicity related increase in mutant frequency, an increase of at least two times the minimum criterion (as defined above), and repeatability of the positive response was required to consider a material mutagenic. A test material was determined to be nonmutagenic if the minimum increase in mutant frequency was not observed for a range of concentrations that extends to toxicity causing 5 to 10% relative suspension growth. If a repeat assay did not confirm an earlier, minimal response, the test material was determined to be nonmutagenic in this assay.
Plates/test: Samples were run in duplicate, with and without metabolic activation.
Activation system: The S-9 fraction from rat (adult male Sprague-Dawley) liver induced with Aroclor 1254.
Media: The cells were maintained in Fischer's mouse leukemia medium supplemented with L-glutamine, sodium pyruvate, and horse serum (10% by volume). Cloning medium consisted of the preceding growth medium with the addition of agar to a final concentration of 0.35% to achieve a semisolid state. Selection medium was cloning medium containing 100 μg/mL of BrdU or 3 μg/mL of TFT.

Reference: Cifone, M.A. 1981. Unpublished report 20989 entitled "Mutagenicity evaluation of UI 81-1 in the mouse lymphoma forward mutation assay" dated November 30, 1981 from Litton Bionetics, Inc., Kensington, MD, for General Electric Plastics, Mt. Vernon, IN

Reliability: (Klimisch Code 1) Valid without restrictions.

16.0 REPEATED DOSE TOXICITY

Species/strain: Rat/Sprague-Dawley Crl:CD®BR
Sex: Female [ ]; Male [ ]; Male/Female [X]; No data [ ]
Route of Administration: Oral Gavage
Exposure period: 13 Weeks
Frequency of treatment: Daily
Post exposure observation period: None
Doses: 0, 0.1, 1.0, and 10 mg/kg/day
Control group: Yes [X]; No [ ]; No data [ ];
Concurrent no treatment [ ]; Concurrent vehicle [X];
Historical [ ]
NOEL: 1.0 mg/kg/day

LOEL: 10 mg/kg/day

Results: *Mortality*: All animals survived to termination of the study.
Clinical signs of toxicity: Findings consisted of cut teeth, malocclusion, tremors, alopecia, lacrimation, and chromodacryorrhea. They were noted sporadically in a small number of animals throughout the treatment groups and control group and were not considered related to compound administration.
Body weight and food consumption: There were no statistical differences between treatment groups and controls for mean body weight or body weight change or any food consumption parameter at any point in the study.
Ophthalmoscopic Examinations: At study initiation there were no observed ophthalmic abnormalities in the animals placed on the study. At study termination conjunctivitis (one mid-dose male) and retinitis (one high-dose female) were observed. The incidence of these findings did not suggest a relationship to treatment.
Hematology/Clinical Chemistry: In the high dose group, decreased eosinophils (males only; 0.2 $\pm$ 0.23 TH/UL% in the control vs. 0.0 $\pm$ 0.07 TH/UL% in the 10 mg/kg/day group) and increased reticulocytes (females only; 1.3 $\pm$ 0.34% RBC in the control vs. 1.6 $\pm$ 0.25% RBC in the 10 mg/kg/day group) were noted. Because of the low magnitude of change and the lack of any other hematological finding, the authors concluded these findings to be spurious and not treatment-related. There were no statistically significant differences in any of the clinical chemistry parameters evaluated.
Gross Pathology: There were a number of gross findings observed at necropsy of which the incidences were sporadic throughout all groups, including the vehicle control. The findings were all of the nature typically seen in this age and strain of rat at the laboratory.
Organ Weight Data: Statistically significant increases were found in the absolute liver (7.08 $\pm$ 0.57 grams versus 8.37 $\pm$ 1.28 grams) and spleen (0.44 $\pm$ 0.08 grams versus 0.55 $\pm$ 0.15 grams) weights of the high dose females versus the vehicle control group. No statistically significant differences were noted for any other absolute or relative organ weight.
Histopathology: No compound related histopathological tissue changes were observed as all findings were of similar frequency across all dose groups and the control group.

Method (Year): OECD 408 (1981)
Rats (10/sex/group) were approximately 7 weeks of age at study start. Males weighed between 240 and 340 grams and females weighed between 168 and 220 grams. All animals were supplied by Charles River Laboratories, Raleigh, N.C., and were housed individually in hanging wire cages with Purina Certified Rodent Chow® #5002 and tap water available *ad libitum*. 0.5% Carboxymethyl cellulose was used as the vehicle. Environmental conditions throughout the study were maintained at a temperature of 72 $\pm$ 6°F, relative humidity of 50 $\pm$ 20%, and light/dark cycle of 12 hours. Opthalmological examination, hematology, clinical biochemistry, gross necropsy, and histopathology

	were performed according to the OECD test guideline. Specific organ weights measured included spleen, liver, kidneys, testes with epididymides, and adrenals. Appropriate statistical analyses were performed on body weight, food consumption, clinical hematology, clinical chemistry, and organ weight data from the same sex of the treated groups and the control group. Statistical significance was designated in this study as $p \leq 0.05$.
GLP:	Yes [X] No [ ] ? [ ]
Test substance:	4-NPI (CAS# 41663-84-7) Supplied by General Electric Plastics, Mt. Vernon, IN Commercial, purity: > 95% 4-NPI (<5% 3-NPI)
Remarks:	Analytical confirmation of dose concentration, homogeneity, and stability were performed.
Reference:	Osheroff, M. 1989. Unpublished report no. HLA 349-434 entitled "Subchronic toxicity study in rats with 4-NPI" dated October 5, 1989 from Hazleton Laboratories America, Inc., Rockville, MD for General Electric Plastics, Pittsfield, MA.
Reliability:	(Klimisch Code 1) Valid without restrictions

Additional References for Repeated Dose Toxicity: Before conducting this 90-day toxicity study, a well-conducted and reliable 28-day study[1] using 5 rats/sex/group was conducted at this same laboratory. Doses were 0, 10, 100, 500, and 1000 mg/kg/day. Animal husbandry, daily clinical observations, ophthalmoscopic examinations, hematology, and clinical biochemistry were conducted as described above. Organ weights were performed on liver, kidneys, and spleen and tissue preservation and histopathological examination were conducted on all gross lesions, kidneys, liver, and spleen. In the 1000 mg/kg/day group 1 male and 3 females died. Statistical evaluation of body weight, body weight gain, and total food consumption values revealed a significant depression for the 1000 mg/kg/day male and female rats. In addition, the 500 mg/kg/day males and females revealed a significant reduction in mean body weights and mean body weight gains throughout much of the experiment. Evaluation of clinical pathology parameters revealed a number of significant changes from control values suggestive of effects on the erythrocytes (decreased numbers of erythrocytes and increased numbers of reticulocytes), and on the kidney and liver of the 100, 500, and 1000 mg/kg/day groups. At necropsy, gross findings included enlarged and darkly discolored spleens (both sexes at 100, 500, and 1000 mg/kg) and darkened kidneys and livers (both sexes: 500 and 1000 mg/kg). Statistically significant organ weight changes included increased absolute and relative spleen weights (both sexes at 100, 500, and 1000 mg/kg); increased liver weights (males at 10 mg/kg and males/females at 100, 500, 1000 mg/kg); and relative kidney weight increases (males at 100 mg/kg and males/females at 500 and 1000 mg/kg). Histopathological examination of the spleen, liver, and kidneys of the 100, 500, and 1000 mg/kg/day groups showed the presence of intracellular pigmentation, characterized as hemosiderin. No unusual histopathological findings were reported for the controls or 10 mg/kg/day groups.

[1] Osheroff, M.R. (1988). Unpublished report no. HLA 349-430 entitled "28-day repeated dose range finding study in rats with 4-NPI" dated June 24, 1988 from Hazleton Laboratories America, Inc., Rockville, MD for General Electric Plastics, Pittsfield, MA.

17.0 REPRODUCTIVE TOXICITY

No studies were found.

18.0 DEVELOPMENTAL TOXICITY/TERATOGENICITY

18.1
Species/strain: Rabbit/New Zealand White
Sex: Female [X]; Male [ ]; Male/Female [ ]; No data [ ]
Route of Administration: Oral gavage
Duration of the test: 29 days
Exposure period: Days 6-19 of gestation
Frequency of treatment: Once daily
Doses: 0, 10, 50, or 200 mg/kg
Control group: Yes [X] (Thalidomide; 150 mg/kg/day); No [ ]; No data [ ]; Concurrent no treatment [ ]; Concurrent vehicle [X] (0.5% Carboxymethylcellulose); Historical [ ]
NOAEL Maternal Toxicity: 50 mg/kg/day
NOAEL teratogenicity: 50 mg/kg/day
Results: *Maternal data*: There were no compound-related deaths during the study. Clinical signs were limited to signs of anorexia (8/16, 6/16, and 10/16 in the 10, 50, and 200 mg/kg groups, respectively, compared to 3/16 in the controls). Other signs and symptoms (soft feces and slight depression) occurred sporadically and did not show dose-related trends. A significant mean maternal body weight loss was seen in the 200 mg/kg group during treatment (gestation days 11-15 and 15-20), when compared to controls. Slight maternal toxicity was evident in the 50 mg/kg group, as seen by the mean body weight loss from study days 6-11 and reduced body weight gain from study days 11-15, when compared to the control group. There were no remarkable changes in absolute body weight or body weight changes in the 10 mg/kg group. There were no treatment-related gross pathological findings.

Pregnancy and fetal data: The results of the cesarean section and litter data are presented below:

Observations	Vehicle Control	Positive Control	10 mg/kg	50 mg/kg	200 mg/kg
# Females Mated	16	16	16	16	16
# Pregnant (%)	15 (94)	11 (69)	14 (88)	16 (100)	16 (100)
# Surviving to Day 29 (%)	14 (100)[a]	11 (100)	14 (100)	14 (88)	14 (93)[a]
# Females Aborting (%)	0 (0)	0 (0)	0 (0)	2 (13)	1 (6)
# Litters Examined	13[b]	9[c]	14	14	14
Mean Data:					

- Corpora Lutea	10.3	10.4	11.8	12.9	12.0
- Implantations	7.8	8.3	6.7	9.4	9.1
- Live Fetuses (%)	7.5 (91)	4.5 (51)	6.4 (95)	8.9 (94)	7.6 (87)
- Dead Fetuses (%)	0.1 (0.7)	0.2 (2)	0.0 (0)	0.0 (0)	0.0 (0)
- Early Resorptions (%)	0.2 (8)	3.5 (45)	0.4 (5)	0.6 (6)	0.5 (4)
- Late Resorptions (%)	0.0 (0)	0.2 (2)	0.0 (0)	0.0 (0)	1.0 (9)*
- Total Resorptions (%)	0.2 (8)	3.6 (46)	0.4 (5)	0.6 (6)	1.5 (13)
- Male Fetuses (%)	4.7 (59)	2.9 (49)	3.6 (56)	4.1 (48)	3.6 (50)
- Implantation Effic. (%)	72	82	59	74	72
- Fetal Wt. (g)	45.7	37.5*	43.2	46.0	44.9
- Male Fetal Wt. (g)	47.0	38.4	43.2	46.2	44.8
- Female Fetal Wt. (g)	42.5	35.7*	40.6	45.0	43.3
# Implants Affected (%)[d]	5 (5)	79 (87)*	6 (6)	12 (9)	27 (21)*
# Fetuses Malformed (%)	1 (1)	37 (76)*	1 (1)	4 (3)	6 (6)
# Litters with Affected Implantations (%)	3 (21)	11 (100)*	5 (36)	6 (43)	9 (64)*
# Litters with Malformed Fetuses (%)	1 (8)	9 (100)*	1 (7)	4 (29)	5 (36)

Footnotes:

a - Excludes one accidental death in the vehicle control and 200 mg/kg groups

b - One litter contained 100% resorptions and no viable fetuses

c - Two litters contained 100% resorptions and no viable fetuses

d - Affected implantations = Malformed fetuses plus nonliving implantations

* - Significant at $p < 0.05$

In the positive control group (thalidomide), there were a number of statistically significant findings (decreased fetal weight, increase number of implants affected, fetuses malformed, and external, visceral, and skeletal malformations). For 4-NPI, there was a significant increase in the number of late resorptions, the incidence of implants affected and the total number of litters with affected implantations in the 200 mg/kg group. There were no other significant findings on any other parameters in any treatment group when compared to the vehicle control. For external and visceral examinations of the 4-NPI treated rabbits, neither the number of fetuses nor the number of litters with malformations was significantly increased when compared to the vehicle control. Following skeletal examinations, the total number of fetuses and litters with skeletal variations was comparable to the vehicle control in all 4-NPI treated groups. Observed skeletal variations included a number of common individual differences in fetal rib count, reduced or delayed ossification of skull bones, sternebrae, pelvic girdle, metacarpals, metatarsals, and phalanges. The incidence of these were not dose-related. The incidence of vertebral anomalies, with or without associated rib anomalies, was increased in the 50 and 200 mg/kg groups, although not significantly. In conclusion, 50 mg/kg/day was considered to be the no observable adverse effect level (NOAEL) for maternal toxicity, fetal viability, and fetal development. Thus, based on this study, 4-NPI is not considered to be a selective developmental toxicant.

Method (Year): U.S. EPA Subdivision F, Series 83-3 (1983) (Similar to OECD 414)

Rabbits (supplied by Hazleton Dutchland, Denver, PA) were housed individually in elevated stainless-steel cages and acclimated to laboratory conditions for 22 days before study initiation. Food and tap water were available *ad libitum*. Ovulation was induced in each female by intravenous injection of 0.25 mL of human chorionic gonadotropin into the marginal ear vein. Each female was artificially inseminated twice; within 55-80 minutes following injection, and again three hours later. The day of insemination was assigned as study day 0. Body weights on day 0 ranged from 3293 to 4409 grams. Rabbits were assigned to 1 of 5 groups (16/group): negative vehicle control (0 mg/kg/day 0.5% carboxymethyl cellulose), positive control (thalidomide; 150 mg/kg/day), and 10, 50, or 200 mg/kg/day of 4-NPI. Females were given 1.0 mL/kg of the appropriate vehicle or dosing suspension by oral intubation on a daily basis beginning on gestation day (gd) 6 and continuing through gd 19 of presumed gestation. The dose administered to each female was based on gd 6 body weights. All animals were examined twice daily for mortality and moribundity, and once daily throughout gestation for obvious indications of toxicity. Body weights were recorded on gd 0, 6, 11, 15, 20, and 29. Females showing signs of abortion or premature delivery were sacrificed band examined grossly for any abnormalities and ovaries examined for the number of corpora lutea, as well as the number of normal and resorbed (early or late) fetuses. On gd 29, all surviving females were sacrificed and the uterus was excised, weighed, and examined for the number and placement of uterine implantation sites, number of live and dead fetuses, number of early- and late-resorbing fetuses, and any abnormalities. Each viable fetus was sacrificed, weighed, sexed, and examined externally for variations and malformations. Each fetus was then subjected to a visceral examination (Staples, 1974 and Wilson, 1965 techniques). Following visceral examination, all fetuses were prepared and stained for skeletal examinations. Statistical analyses were performed on maternal and fetal body weights and incidence data, such as clinical signs, percent males/litter, fetal viability, resorptions, and malformations.

GLP: Yes [X] No [ ] ? [ ]

Test substance: 4-NPI (CAS# 41663-84-7) Supplied by General Electric Plastics, Mt. Vernon, IN
Commercial, purity: > 95% 4-NPI (<5% 3-NPI)

Remarks: This protocol differed from current OECD 414 guidelines only with respect to number of animals per group (16 versus 20), and analytical chemistry was not performed.

Reference: Cox, R. H. Unpublished report no. HLA 349-325 entitled "Rabbit teratology study with 4-NPI" dated March 6, 1986 from Hazleton Laboratories America, Inc., Vienna, VA for General Electric Plastics, Pittsfield, MA.

Reliability: (Klimisch Code 1) Valid without restrictions

18.2

Species/strain: Rat/Sprague-Dawley Crl:CD®BR

Sex: Female [X]; Male [ ]; Male/Female [ ]; No data [ ]

Route of Administration: Oral gavage

Duration of the test: 20 days
Exposure period: Days 6-15 of gestation
Frequency of treatment: Once daily
Doses: 0, 10, 50, or 200 mg/kg
Control group: Yes [X] (Vitamin A; 100,000 IU); No [ ]; No data [ ]; Concurrent no treatment [ ]; Concurrent vehicle [X] (0.5% Carboxymethylcellulose); Historical [ ]
NOAEL Maternal Toxicity: 10 mg/kg/day
NOAEL teratogenicity: 50 mg/kg/day
Results: *Maternal data*: All control and test substance animals survived to day 20 of gestation. During the treatment phase, treatment-related clinical signs (thinness, hunched posture, rough hair coat, urine stains, anorexia, sensitive to touch, squinted eyes, rhinorrhea, and vaginal discharge) were only observed in the 200 mg/kg group. A statistically significant reduction in body weight was observed at days 8, 12, 16, and 20 in the 200 mg/kg group. Body weight change was reduced in the 50 mg/kg group at days 6-8 and in the 200 mg/kg group at days 8-12, 6-16, 6-20, and 0-20, when compared to the vehicle control. Food consumption was also reduced in the 50 and 200 mg/kg groups on days 6-8 and 8-12 and days 12-16 and 0-20, respectively. The mean gravid uterine weight was significantly decreased in the 200 mg/kg group, when compared to the negative control group. Gross pathology was limited to one animal in the 200 mg/kg group with tan, green material in the uterine horns and one animal in the 50 mg/kg with dilated renal pelvises.

Pregnancy and fetal data: The results of the cesarean section and litter data are presented below:

Observations	Vehicle Control	Positive Control	10 mg/kg	50 mg/kg	200 mg/kg
# Females Mated	24	24	24	24	24
# Pregnant (%)	23 (96)	23 (96)	23 (96)	24 (100)	23 (96)
# Surviving to Day 20 (%)	24 (100)	24 (100)	24 (100)	24 (100)	24 (100)
# Females Aborting (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Mean Data:					
- Corpora Lutea	16.9	17.0	17.0	17.9	16.3
- Implantations	14.9	13.7	14.9	14.9	13.0
- Live Fetuses (%)	14.2 (95)	10.2 (74)	14.3 (96)	14.3 (96)	10.0 (71)
- Dead Fetuses (%)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
- Early Resorptions (%)	0.7 (5)	3.3 (24)	0.6 (4)	0.7 (4)	2.6 (26)
- Late Resorptions (%)	0.0 (0)	0.2 (2)	0.1 (0)	0.0 (0)	0.3 (3)
- Total Resorptions (%)	0.7 (5)	3.6 (26)	0.7 (4)	0.7 (4)	3.0 (29)*
- Male Fetuses (%)	6.8 (48)	5.1 (50)	7.0 (49)	7.1 (50)	5.1 (39)
- Implantation Effic. (%)	89	82	89	86	79*
- Fetal Wt. (g)	3.5	3.0	3.5	3.5	2.8*
- Male Fetal Wt. (g)	3.6	3.1	3.6	3.6	2.8*
- Female Fetal Wt. (g)	3.4	3.0	3.4	3.5	2.7*

Footnotes:

* - Significant at $p < 0.05$

For 4-NPI, *in utero* toxicity was indicated by a statistically significant increase in the percent total resorptions per litter and a significant decrease in mean male, female, and combined male and female fetal body weight values when compared to the negative control group. For fetal external observations, there were no external variation noted in any 4-NPI treated group. For malformations, the 200 mg/kg group showed four fetuses with small or absent pinnae and one with a short tail. The only external malformation observed in the control group was a fetus with anophthalmia. There were no external malformations in the 10 or 50 mg/kg groups. No soft-tissue malformations were observed in any of the 4-NPI treated groups. Soft-tissue variations (significant only in the 200 mg/kg group) included small renal papilla, no renal papilla, and moderately and severely dilated ureters. No skeletal malformations were observed in fetuses in any of the 4-NPI treated groups. Skeletal variations (primarily incomplete ossification of pubis, vertebrae, or sternebrae) were significantly above the control group in the 50 and 200 mg/kg groups. In the absence of other signs of delayed development, these variations were not considered to be a treatment-related effect. For the positive control group (Vitamin A), the percents of early and total resorptions, implantation efficiency, and fetal body weight were reduced from the negative group. External malformations (exencephaly with associated eye anomalies and tongue protrusion), soft-tissue malformations (cleft palate), and skeletal malformations were significantly increased in the positive control group. In conclusion, 10 mg/kg/day was considered to be the no observable adverse effect level (NOAEL) for maternal toxicity and 50 mg/kg was considered to be the NOAEL for fetal viability and fetal development. Thus, based on this study, 4-NPI is not considered to be a selective developmental toxicant.

Method (Year): U.S. EPA Subdivision F, Series 83-3 (1983) (Similar to OECD 414)

Rats (supplied by Charles River Breeding Laboratories, Portage, MI) were housed in hanging wire stainless-steel cages. Following a 12 day acclimation period, one male and one female rat were housed per cage on breeding racks. Following confirmation of mating, females were uniquely identified and individually housed. The day of observation of sperm or copulatory plug was designated as day 0 of gestation (gd 0). Food and tap water were available *ad libitum*. Rats were approximately 10 to 12 weeks of age at study initiation and weighed 223.7 $\pm$ 12.71 grams. Rats were assigned to 1 of 5 groups (24/group); negative vehicle control (0 mg/kg/day - 0.5% carboxymethyl cellulose), positive control (Vitamin A drops – 2.0 ml of 100,000 IU on gd 8), and 10, 50, or 200 mg/kg/day of 4-NPI. Females were given 10.0 ml/kg of the appropriate vehicle or dosing suspension by oral intubation on a daily basis beginning on gd 6 and continuing through gd 15 of presumed gestation. The dose administered to each female was based on gd 6 body weights. All animals were examined twice daily for mortality and moribundity, and once daily throughout gestation for obvious indications of toxicity. Body weights were recorded on gd 0, 6, 8, 12, 16, and 20. Food consumption was also measured at each

	interval. On gd 20, all surviving females were sacrificed and the uterus was excised, weighed, and examined for the number and placement of uterine implantation sites, number of live and dead fetuses, number of early and late resorbing fetuses, and any abnormalities. The ovaries from all females were examined for the number of corpora lutea. Each viable fetus was sacrificed, sexed, weighed, and examined externally for variations and malformations. Each fetus was then subjected to a visceral examination (Staples, 1974 and Wilson, 1965 techniques). Following visceral examination, all fetuses were prepared and stained for skeletal examinations. Statistical analyses were performed on maternal and fetal body weights and incidence data, such as clinical signs, percent males/litter, fetal viability, resorptions, and malformations.
GLP:	Yes [X] No [ ] ? [ ]
Test substance:	4-NPI (CAS# 41663-84-7) Supplied by General Electric Plastics, Mt. Vernon, IN Commercial, purity: > 95% 4-NPI (<5% 3-NPI)
Remarks:	This protocol differed from current OECD 414 guidelines only with respect to duration of treatment during gestation (gd 6-15 versus entire period of gestation).
Reference:	Morseth, S. L. Unpublished report no. HLA 349-264 entitled "Rat teratology study with 4-NPI" dated April 24, 1987 from Hazleton Laboratories America, Inc., Vienna, VA for General Electric Plastics, Pittsfield, MA.
Reliability:	(Klimisch Code 1) Valid without restrictions